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(54) Title: ALPHAVIRUS VECTORS FOR PARAMYXOVIRUS VACCINES

(57) Abstract

A DNA vector comprises a first DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complement of complete alphavirus DNA genome replication regions, and a second DNA sequence encoding a paramyxovirus protein, particularly a respiratory syncytial virus fusion (RSV F) protein or a RSV F protein fragment that generates antibodies that specifically react with RSV F protein, the first and second DNA sequences being under the transcriptional control of a promoter, preferably a cytomegalovirus promoter, which may include Intron A. Such vectors also contain a further nucleotide sequence located between the promoter sequence and the alphavirus sequence to enhance the immunoprotective ability of the RSV F protein when expressed *in vivo*. Such DNA vectors may be used to immunize a host against disease caused by infection with RSV or other paramyxovirus, including a human host, by administration thereto, and may be formulated as immunogenic compositions with pharmaceutically-acceptable carriers for such purposes. Such vectors also may be used to produce antibodies for detection of RSV or other paramyxovirus infection in a sample.

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TITLE OF INVENTIONALPHAVIRUS VECTORS FOR PARAMYXOVIRUS VACCINES

5

FIELD OF INVENTION

The present invention relates to the field of paramyxoviridae vaccines and is particularly concerned with vaccines comprising DNA encoding the fusion (F) protein of respiratory syncytial virus (RSV) in an alphavirus vector.

BACKGROUND OF THE INVENTION

Human respiratory syncytial virus (RSV) has been identified as a major pathogen responsible for severe respiratory tract infections in infants, young children and the institutionalized elderly (refs. 1,2,3,4 - throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). Global mortality and morbidity figures indicate that there is an urgent need for an efficacious RSV vaccine (refs. 5,6). In the USA alone, approximately 100,000 children are hospitalized annually with severe cases of pneumonia and bronchiolitis resulting from an RSV infection. Inpatient and ambulatory care for children with RSV infections has been estimated to cost in excess of \$340 million each year in the USA. The World Health Organization (WHO) and the National Institute of Allergy and Infectious Disease (NIAID) vaccine advisory

committees have ranked RSV second only to HIV for vaccine development. Both the annual morbidity and mortality figures as well as the staggering health care costs for managing RSV infections have provided the incentive for aggressively pursuing the development of efficacious RSV vaccines. However, such a vaccine is still not available.

Formalin-inactivated (FI-RSV) and live attenuated RSV vaccines have failed to demonstrate efficacy in clinical trials (refs. 7,8,9,10). Moreover, the formalin-inactivated RSV vaccine caused enhanced disease in some children following exposure to wild-type RSV (refs. 7,8,9,10). Elucidation of the mechanism(s) involved in the potentiation of RSV disease is important for the design of safe RSV vaccines, especially for the seronegative population. Recent experimental evidence suggests that an imbalance in cell-mediated responses may contribute to immunopotential. Enhanced histopathology observed in mice that were immunized with the FI-RSV and challenged with virus could be abrogated by depletion of CD4+ cells or both interleukin-4 (IL-4) and IL-10.

The RSV fusion (F) glycoprotein is one of the major immunogenic proteins of the virus. This envelope glycoprotein mediates both fusion of the virus to the host cell membrane and cell-to-cell spread of the virus (ref. 1). The F protein is synthesized as a precursor (F_0) molecule which is proteolytically cleaved to form a disulphide-linked dimer composed of the N-terminal F_2 and C-terminal F_1 moieties (ref. 11). The amino acid sequence of the F protein is highly conserved among RSV

subgroups A and B and is a cross-protective antigen (refs. 6,12). In the baculovirus expression system, a truncated secreted version of the RSV F protein has been expressed in *Trichoplusia ni* insect cells (ref. 13). The recombina⁵nt protein was demonstrated to be protective in the cotton rats (ref. 13).

Studies on the development of live viral vaccines and glycoprotein subunit vaccines against parainfluenza virus infection are being pursued. Clinical trial results with a formalin-inactivated PIV types 1,2,3 vaccine demonstrated that this vaccine was not efficacious (refs. 14, 15, 16). Further development of chemically-inactivated vaccines was discontinued after clinical trials with a formalin-inactivated RSV vaccine demonstrated that not only was the vaccine not effective in preventing RSV infection but many of the vaccinees who later become infected with RSV suffered a more serious disease. Most of parainfluenza vaccine research has focused on candidate PIV-3 vaccines (ref. 17) with significantly less work being reported for PIV-1 and PIV-2. Recent approaches to PIV-3 vaccines have included the use of the closely related bovine parainfluenza virus type 3 and the generation of attenuated viruses by cold-adaptation of the virus (refs. 18, 19, 20, 21).

Another approach to parainfluenza virus type 3 vaccine development is a subunit approach focusing on the surface glycoproteins hemagglutinin-neuraminidase (HN) and the fusion (F) protein (refs. 22, 23, 24). The HN antigen, a typical type II glycoprotein, exhibits both haemagglutination and neuraminidase activities and

is responsible for the attachment of the virus to sialic acid containing host cell receptors. The type I F glycoprotein mediates fusion of the viral envelope with the cell membrane as well as cell to cell spread of the virus. It has recently been demonstrated that both the HN and F glycoproteins are required for membrane fusion. The F glycoprotein is synthesized as an inactive precursor (F) which is proteolytically cleaved into disulfide-linked F2 and F1 moieties. While the HN and F proteins of PIV-1, -2 and -3 are structurally similar, they are antigenically distinct. Neutralizing antibodies against the HN and F proteins of one of PIV type are not cross-protective. Thus, an effective PIV subunit vaccine must contain the HN and F glycoproteins from the three different types of parainfluenza viruses. Antibody to either glycoprotein is neutralizing *in vitro*. A direct correlation has been observed between the level of neutralizing antibody titres and resistance to PIV-3 infections in infants. Native subunit vaccines for parainfluenza virus type 3 have investigated the protectiveness of the two surface glycoproteins. Typically, the glycoproteins are extracted from virus using non-ionic detergents and further purified using lectin affinity or immunoaffinity chromatographic methods. However, neither of these techniques may be entirely suitable for large scale production of vaccines under all circumstances. In small animal protection models (hamsters and cotton rats), immunization with the glycoproteins was demonstrated to prevent infection with live PIV-3 (refs. 25, 26, 27, 28, 29).

The HN and F glycoproteins of PIV-3 have also been produced using recombinant DNA technology. HN and F glycoproteins have been produced in insect cells using the baculovirus expression system and by use of vaccinia virus and adenovirus recombinants (refs. 30, 31, 32, 33, 34). In the baculovirus expression system, both full-length and truncated forms of the PIV-3 glycoproteins as well as a chimeric F-HN fusion protein have been expressed. The recombinant proteins have been demonstrated to be protective in small animal models (see WO91/00104, US Application No. 07/773,949 filed November 29, 1991, assigned to the assignee hereof).

Semliki Forest virus (SFV) is a member of the Alphavirus genus in the Togaviridae family. The mature virus particle contains a single copy of a ssRNA genome with a positive polarity that is 5'-capped and 3'-polyadenylated. It functions as an mRNA and naked RNA can start an infection when introduced into cells. Upon infection/transfection, the 5' two-thirds of the genome is translated into a polyprotein that is processed into the four nonstructural proteins (nsP1 to 4) by self cleavage. Once the ns proteins have been synthesized they are responsible for replicating the plus-strand (42S) genome into full-length minus strands (ref. 14). These minus-strands then serve as templates for the synthesis of new plus-strand (42S) genomes and the 26S subgenomic mRNA (ref. 14). This subgenomic mRNA, which is colinear with the last one-third of the genome, encodes the SFV structural proteins.

In 1991 Liljestrom and Garoff (ref. 15) designed a series of expression vectors based on the SFV cDNA

replicon. These vectors had the virus structural protein genes deleted to make the way for heterologous inserts, but preserved the nonstructural coding region for production of the nsP1 to 4 replicase complex.

5 Short 5' and 3' sequence elements required for RNA replication were also preserved. A polylinker site was inserted downstream from the 26S promoter followed by translation stop sites in all three frames. An SpeI site was inserted just after the 3' end of the SFV cDNA
10 for linearization of the plasmid for use *in vitro* transcription reactions.

Injection of SFV RNA encoding a heterologous protein have been shown to result in the expression of the foreign protein and the induction of antibody in a
15 number of studies (refs. 16,17). The use of SFV RNA inoculation to express foreign proteins for the purpose of immunization would have several of the advantages associated with plasmid DNA immunization. For example, SFV RNA encoding a viral antigen may be introduced in
20 the presence of antibody to that virus without a loss in potency due to neutralization by antibodies to the virus. Also, because the protein is expressed *in vivo* the protein should have the same conformation as the protein expressed by the virus itself. Therefore,
25 concerns about conformational changes which could occur during protein purification leading to a loss in immunogenicity, protective epitopes and possibly immunopotential, could be avoided by plasmid DNA immunization.

30 In copending US Patent Application No. 08/476,397 filed June 7, 1995, assigned to the assignee hereof and

the disclosure of which is incorporated herein by reference (WO96/040945), there is described reference the use of plasmid vectors containing RSV F protein-encoding DNA for DNA immunization against RSV infection.

5 In copending United States Patent Application No. 08/896,500 filed July 18, 1997, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference, there is described the use of plasmid vectors containing RSV G protein-encoding DNA
10 for DNA immunization against RSV infection.

In my copending United States Patent Application No. 08/923,558, filed September 4, 1997, assigned to the assignee hereof and the disclosure of which is incorporated by reference, I describe a DNA vector using
15 an alphavirus vector, including Semliki Forest virus vector, containing a DNA sequence encoding a paramyxovirus protein, specifically RSV-F, for making an RNA transcript for immunization.

In WO95/27044, the disclosure of which is
20 incorporated herein by reference, there is described the use of alphavirus cDNA vectors based on cDNA complementary to the alphavirus RNA sequence. Once transcribed from the cDNA under transcriptional control of a heterologous promoter, the alphavirus RNA is able
25 to self-replicate by means of its own replicase and thereby amplify the copy number of the transcribed recombinant RNA molecules.

Infection with RSV leads to serious disease. It would be useful and desirable to provide improved
30 vectors for *in vivo* administration of immunogenic preparations, including vaccines, for protection against

disease caused by RSV and other paramyxoviruses. In particular, it would be desirable to provide vaccines that are immunogenic and protective in humans, including seronegative infants, that do not cause disease enhancement (immunopotentialiation).

SUMMARY OF THE INVENTION

The present invention provides novel immunogenic materials and immunization procedures based on such novel materials for immunizing against disease caused by respiratory syncytial virus. In particular, the present invention is directed towards the provision of DNA vaccines against disease caused by infection with paramyxoviridae.

In accordance with one aspect of the present invention, there is provided a vector, comprising a first DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complement of complete alphavirus RNA genome replication regions to permit *in vivo* replication; a second DNA sequence encoding a paramyxovirus protein or a protein fragment that generates antibodies that specifically react with the paramyxovirus protein, the second DNA sequence being inserted into a region of the first DNA sequence which is non-essential for replication; the first and second DNA sequences being under transcriptional control of a promoter; and a third DNA sequence located adjacent the second DNA sequence to enhance the immunoprotective ability of the paramyxovirus protein when expressed *in vivo* from the vector in a host.

The paramyxovirus protein may be selected from the group consisting of a parainfluenza virus (PIV) and a respiratory syncytial virus (RSV). The PIV protein may be from PIV-1, PIV-2, PIV-3 or PIV-4, particularly the
5 HN and F glycoproteins of PIV-3. The RSV protein particularly may be the F or G glycoprotein of RSV.

The second DNA sequence may encode a full length RSV F protein, or may encode a RSV F protein lacking the transmembrane anchor and cytoplasmic tail. The
10 lack of the coding region for the transmembrane anchor and cytoplasmic tail results in a secreted form of the RSV F protein. Alternatively, as described in the aforementioned U.S. Patent Application 08/896,500, the second DNA sequence may encode the full-length RSV-G
15 protein or a truncated RSV G protein lacking a transmembrane region, resulting in a secreted form of the protein.

The alphavirus preferably is a Semliki Forest virus and the first DNA sequence is the Semliki Forest viral
20 sequence contained in plasmid pSFVI.

The third nucleotide sequence may comprise a pair of splice sites to prevent aberrant mRNA splicing, *in vivo*, whereby substantially all transcribed mRNA from the vector upon administration encodes the RSV protein.
25 Such third nucleotide sequence is preferably located between the first nucleotide sequence and the promoter sequence. Such third nucleotide sequence may be that of rabbit β -globin intron II, as shown in Figure 8 of copending U.S. Patent Application No. 08/476,397 (WO
30 96/040945).

The promoter sequence may be an immediate early cytomegalovirus (CMV) promoter. The human cytomegalovirus Intron A sequence may be provided downstream of the promoter and upstream of the third
5 nucleotide sequence.

A vector encoding the F protein and provided in accordance with one embodiment of the invention may be specifically pMP44, having the identifying characteristics shown in Figure 1D.

10 The vectors provided herein may be used to immunize a host against RSV infection or disease by *in vivo* expression of RSV F protein or RSV G protein, which may lack a transmembrane region, or other paramyxovirus protein, following administration of the vectors. In
15 accordance with a further aspect of the present invention, therefore, there is provided a method of immunizing a host against disease caused by infection with respiratory syncytial virus or other paramyxovirus, which comprises administering to the host an effective
20 amount of a vector provided herein.

The present invention also includes a novel method of using a gene encoding an RSV F or G protein or a fragment of an RSV or G protein capable of generating antibodies which specifically react with RSV F or G
25 protein to protect a host against disease caused by infection with respiratory syncytial virus, which comprises isolating the gene; operatively linking said gene to a DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the
30 complement of complete alphavirus RNA genome replication regions in a region of said DNA sequence which is non-

essential for replication to form a vector wherein said gene and DNA sequence are under transcriptional control of a promoter; operatively linking the gene to an immunoprotection enhancing sequence to produce an enhanced immunoprotection by the RSV F or G protein in the host, preferably by introducing the immunoprotection enhancing sequence between the control sequence and the alphavirus sequence; and introducing the vector into the host. A corresponding procedure may be used for other paramyxoviridae.

In addition, the present invention includes a method of producing a vaccine for protection of a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises isolating a first DNA sequence encoding an RSV or G protein, from which the transmembrane anchor and cytoplasmic tail may be absent; operatively linking said first DNA sequence to a second DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complete alphavirus genome replication regions in a region of said second DNA sequence which is non-essential for replication to form a vector wherein said first and second DNA sequences are under transcriptional control of a promoter; operatively linking the first nucleotide sequence to a third nucleotide sequence to enhance the immunoprotective ability of the RSV F or G protein when expressed *in vivo* from the vector in a host; and formulating the vector as a vaccine for *in vivo* administration. A corresponding procedure may be used for other paramyxoviridae.

The present invention further includes a vaccine for administration to a host, including a human host, produced by the method as well as immunogenic compositions comprising an immunoeffective amount of the
5 vectors described herein.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1A to 1B show a schematic of a procedure of assembly of vector pMP44;

Figures 2A to 2B show a schematic of a procedure of
10 assembly of vector pMP44;

Figures 3A to 3E contain the nucleotide sequence of plasmid pMP44 (SEQ ID NO:1);

Figure 4 shows the anti-RSV F titres in sera from mice taken 4 weeks after priming and 2 weeks after
15 boosting;

Figure 5 shows the nucleotide sequence for a synthetic oligonucleotide coding for the hepatitis delta ribozyme (SEQ ID no; 2,3); and

Figures 6A to 6C show the nucleotide sequence for
20 the SFV EcoRV-SpeI fragment ligated to the ribozyme of Figure 5 (SEQ ID no: 4).

GENERAL DESCRIPTION OF INVENTION

As described above, the present invention, in general, relates to protection of hosts against disease caused by infection by paramyxovirus by DNA immunization using DNA vectors. In particular, the invention is concerned with protection of hosts against disease caused by infection by respiratory syncytial virus (RSV), although not specifically limited thereto. The description which follows refers specifically to employing DNA sequences encoding RSV F or G protein and fragments thereof which generate antibodies which specifically react with RSV F or G protein.

In this application, the terms "RSV F protein" and "RSV G protein" are used to define a full-length RSV F or G protein, including proteins having variations in their amino acid sequences including those naturally occurring in various strain of RSV and those introduced by PCR amplification of the encoding gene while retaining the immunogenic properties, a secreted form of the RSV F or G protein lacking a transmembrane anchor and cytoplasmic tail, as well as fragments capable of generating antibodies which specifically react with RSV F or G protein and functional analogs. In this application, a first protein is a "functional analog" of a second protein if the first protein is immunologically related to and/or has the same function as the second protein. The functional analog may be, for example, a fragment of the protein or a substitution, addition or deletion mutant thereof.

A vector is constructed to contain a first DNA sequence which is complementary to at least part of an

alphavirus RNA genome, specifically Semliki Forest virus, and having the complement of complete alphavirus RNA genome replication regions to permit replication *in vivo*. A second DNA sequence encoding the RSV F or G protein is inserted into a region of the first DNA sequence which is non-essential for replication. The first and second DNA sequences are under transcriptional control of a promoter to permit expression of the RSV protein in a host immunized with the vector.

10 The promoter sequence may be the immediately early cytomegalovirus (CMV) promoter. This promoter is described in ref. 36. Any other convenient promoter may be used, including constitutive promoters, such as, Rous Sarcoma Virus LTRs, and inducible promoters, such as
15 metallothionine promoter, and tissue specific promoters.

The recombinant vector may include a third nucleotide sequence located adjacent the alphavirus sequence to enhance the immunoprotective ability of the RSV F or G protein when expressed *in vivo* in a host.

20 Such enhancement may be provided by increased *in vivo* expression, for example, by increased mRNA stability, enhanced transcription and/or translation. This additional sequence preferably is located between the promoter sequence and the alphavirus sequence.

25 This enhancement sequence may comprise a pair of splice sites to prevent aberrant mRNA splicing during transcription so that substantially all transcribed mRNA is intact alphavirus RNA encoding a gene of interest, for example, an RSV F protein. Specifically, rabbit β -globin Intron II sequence may provide such splice sites,
30 as also described in ref. 37.

Additional enhancement may be obtained by, including an additional DNA sequence between the promoter and the enhancer sequence. Such additional DNA sequence may comprise the immediate early
5 cytomegalovirus Intron A sequence.

The vectors provided herein, when administered to an animal, effect *in vivo* RSV F protein expression, as demonstrated by an antibody response in the animal to which it is administered and the conferring of
10 protection. As may be seen from the results detailed in the Examples below, the DNA vectors produced a high anti-F IgG antibody titre and confer protection.

In comparison to the vectors described in the aforementioned U.S. Patent Application nos. 08/476,397
15 and 08/896,500, the vectors described herein provide a protective immune response using a lower dose and less time. In comparison to the vectors described in the aforementioned U.S. Patent Application nos. 08/923,558, 08/896,550 and 08/476,397 using native RSV F, the
20 vectors described herein produce protective immune response in the absence of pretreatment of the animal model with cardiotoxin, a material known to increase the uptake of DNA and enhance the immune response.

The vector provided herein may also comprise a
25 fourth nucleotide sequence encoding a further antigen from RSV, an antigen from at least one other pathogen or at least one immunomodulating agent, such as cytokine. Such vector may contain said fourth nucleotide sequence in a chimeric or a bicistronic structure. Alternatively,
30 vectors containing the fourth nucleotide sequence may be

separately constructed and coadministered to a host, with the DNA vector provided herein.

In addition, there may be provided at the 3'-end of the Simliki Forest virus segment, a hepatitis delta virus ribosyme sequence to ensure proper *in vivo* cleavage at the 3'-end of the Simliki Forest virus sequence. Any other convenient sequence may be employed to achieve this effect.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of RSV infections. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from the RSV F or RSV G genes and other paramyxovirus genes and vectors as disclosed herein. The vaccine elicits an immune response in a subject which includes the production of anti-F or anti-G antibodies. Immunogenic compositions, including vaccines, containing the DNA vector may be prepared as injectables, in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The nucleic acid may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a nucleic acid liposome (for example, as described in WO 93/24640, ref. 38) or the DNA vector may be associated with an adjuvant, as described in more detail below. Liposomes comprising cationic lipids interact spontaneously and rapidly with

polyanions such as DNA and RNA, resulting in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an
5 intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal compartment. Published PCT application WO 94/27435 describes compositions for genetic immunization comprising cationic lipids and polynucleotides. Agents which
10 assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other transfection facilitating agents, may advantageously be used.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable
15 time-release particles. Thus, U.S. Patent 5,151,264 describes a particulate carrier of a phospholipid/glycolipid/polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety
20 of molecules having biological activity in one of the layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50
25 poly (DL-lactideco-glycolide). Other polymers for encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co-glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-
30 hydroxybutyric acid), and polyanhydrides.

Published PCT application WO 91/06282 describes a delivery vehicle comprising a plurality of bioadhesive microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This delivery vehicle is particularly intended for the uptake of vaccine across the nasal mucosa. The delivery vehicle may additionally contain an absorption enhancer.

The RSV F or G genes and vectors may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may include, water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously, intravenously, intradermally or intramuscularly, possibly following pretreatment of the injection site with a local anaesthetic. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkalene glycols or triglycerides. Oral formulations may include normally employed incipients,

such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate.

The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize the RSV F protein and antibodies thereto, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of about 1 μ g to about 1 mg of the RSV F or G genes and vectors. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations.

The dosage may also depend on the route of administration and will vary according to the size of the host. A vaccine which protects against only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

In particular embodiments of the present invention, the vector comprising a first nucleotide sequence

encoding an F or G protein of RSV may be delivered in conjunction with a targeting molecule to target the vector to selected cells including cells of the immune system.

5 The DNA vectors may be delivered to the host by a variety of procedures, for example, Tang et al. (ref. 39) disclosed that introduction of gold microprojectiles coated with DNA encoding bovine growth hormone (BGH) into the skin of mice resulted in production of anti-BGH
10 antibodies in the mice, while Furth et al. (ref. 40) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

2. **Immunoassays**

 The RSV F or G genes and vectors of the present
15 invention are useful as immunogens for the generation of anti-F or anti-G antibodies for use in immunoassays, including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art. In ELISA assays, the
20 vector first is administered to a host to generate antibodies specific to the RSV F or G protein or other paramyxovirus protein. These RSV F- or G-specific antibodies are immobilized onto a selected surface, for example, a surface capable of binding the antibodies,
25 such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed antibodies, a nonspecific protein such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample may
30 be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the

immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to 4 hours, at temperatures such as of the order of about 20° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test sample and the bound RSV F specific antibodies, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined.

Biological Deposits

Certain vectors that contain the gene encoding RSV F protein and referred to herein have been deposited with the American Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A., pursuant to the Budapest Treaty and prior to the filing of this application.

Samples of the deposited plasmids will become available to the public upon grant of a patent based upon this United States patent application and all restrictions on access to the deposits will be removed

at that time. Non-viable deposits will be replaced. The invention described and claimed herein is not to be limited in scope by plasmids deposited, since the deposited embodiment is intended only as an illustration
5 of the invention. Any equivalent or similar plasmids that encode similar or equivalent antigens as described in this application are within the scope of this invention.

Deposit Summary

10	<u>Plasmid</u>	<u>ATCC Designation</u>	<u>Date Deposited</u>
	pMP37	97905	Feb. 27, 1997
	pMP42		

EXAMPLES

The above disclosure generally describes the
15 present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and
20 substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

25 Methods of molecular genetics, protein biochemistry and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

EXAMPLE 1

This Example describes a scheme for construction of a Semliki Forest Virus (SFV) DNA expression vector containing a truncated RSV F gene as outlined in Figures 1A to 1B.

Plasmid VR1012 was restricted with PstI and then made blunt-ended with T4 DNA polymerase. The β -globin Intron II was excised out of vector pSG5 (Stratagene) and ligated into plasmid VR1012 to generate plasmid pIIE. Plasmid pIIE was then restricted with SalI and EcoRV and ligated to a PCR fragment having the nucleotide sequence:

TCGACATGGCGGATGTGTGACATACACGACGCCAAAAGATTTTGTTCAGCT
CCTGCCACCTCCGCTACGCGAGAGATTAACCAACCCACGATGGCCGCCAAAGT
GCATGTTGATATTGAGGCTGACAGCCCATTCAAGTCTTGCAGAAGGCA
TTTCCGTCGTTGAGGTGGAGTCATTGCAGGTCACACCAAATGACCATGCAA
ATGCCAGAGCATTTTCGCACCTGGCTACCAAATTGATCGAGCAGGAGACTGA
CAAAGACACACTCATCTTGGAT (SEQ ID no: 7) generated from
pSFVI with primers SAL-SFV having the nucleotide
sequence 5'-TCCACCTCCAAGATATCCAAGATGAGTGTG (SEQ ID no:
5) and ECO-SFV having the nucleotide sequence 5'-
TCCACCTCCAAGATATCCAAGATGAGTGTG (SEQ ID no: 6). The
resulting plasmid pMP38 was then restricted with EcoRV
and BamHI and then dephosphorylated. Plasmid pSFV1
link (see copending application no. _____ (b/o
1038-766)) was then restricted with SpeI and ligated to
the hepatitis delta ribozyme (Fig. 5, SEQ ID nos: 2 and
3). The ligation reaction was then restricted with
EcoRV to release most of the SFV-RSVF plus ribozyme
fragment. This fragment was then ligated to
EcoRV/BamHI restricted pMP38 to produce pMP41.

Example 2

This Example describes an alternative scheme for constructing plasmid pMp44 as outlined in Figure 2.

Plasmid VR1012 was restricted with PstI and then
5 made blunt-ended with T4 DNA polymerase. The β -globin
Intron II was excised out of vector pSG5 (Stratagene)
and ligated into plasmid VR1012 to generate plasmid
pIIE. Plasmid pIIE was then restricted with SalI and
EcoRV and ligated to a PCR fragment having the
10 nucleotide sequence:

TCGACATGGCGGATGTGTGACATACACGACGCCAAAAGATTTTGTTCAGCT
CCTGCCACCTCCGCTACGCGAGAGATTAACCACCCACGATGGCCGCCAAAGT
GCATGTTGATATTGAGGCTGACAGCCCATTCATCAAGTCTTTGCAGAAGGCA
TTTCCGTCGTTGAGGTGGAGTCATTGCAGGTCACACCAAATGACCATGCAA
15 ATGCCAGAGCATTTTCGCACCTGGCTACCAAATTGATCGAGCAGGAGACTGA
CAAAGACACACTCATCTTGGAT (SEQ ID no: 7) generated from
pSFVI with primers SAL-SFV having the nucleotide
sequence 5'-TCCACCTCCAAGATATCCAAGATGAGTGTG (SEQ ID no:
5) and ECO-SFV having the nucleotide sequence 5'-
20 TCCACCTCCAAGATATCCAAGATGAGTGTG (SEQ ID no: 6). The
resulting plasmid pMP38 was then restricted with EcoRV
and BamHI and then dephosphorylated. Plasmid pSFV1
link (see copending application no. _____ (b/o
1038-766)) was then restricted with SpeI and ligated to
25 the hepatitis delta ribozyme (Fig. 5, SEQ ID nos: 2 and
3).

The ligation reaction product was then restricted
with EcoRV to release the SFV replicon plus the
ribozyme having the nucleotide sequence as outlines in
30 Figures 6A to 6C. This fragment was then ligated to
the EcoRV/BamHI restricted pMP38 to produce pMP42. The

RSV F gene fragment was released from pMP37 by restriction with BamHI, and this fragment was ligated into the BamHI site of pMP42 to produce pMP44. The nucleotide sequence of pMP44 is shown in Figures 3A to 3E.

EXAMPLE 3

This Example describes the immunization of mice with pMP44 and the immunogenicity results obtained.

BALB/C mice were immunized with plasmid pMP44 by the intramuscular (i.m.) route. The anterior tibialts muscles of six BALB/C mice were bilaterally injected with 2 x 100 μ g of plasmid pMP44. This amount is equivalent to approximately 94 μ g of a conventional vector, based on copy number. These mice were boosted in an identical manner 4 weeks later. The control group was immunized with 2 x 25 μ g of SFV-RSV F RNA as described in my aforementioned United States Application No. 08/923,558, except that the muscles were not pre-treated with cardiotoxin. The immunization protocol is set forth in the following Table I:

Table 1 Immunization protocol

Group	Prime	Route of Inoculation	Boost	Route of Inoculation
25	1 SFV-RSVF RNA ¹	Intramuscular	SFV-RSVF RNA ¹	Intramuscular
	2 pMP44 DNA ²	Intramuscular	pMP44DNA ²	Intramuscular

Mice were inoculated with:

1. 25 μ g of RNA was injected into each hind leg muscle in 50 μ L of PBS
2. 100 μ g of DNA was injected into each hind leg muscle in 50 μ L of PBS

Sera was obtained from the mice at 4 and 6 weeks. Anti-RSV F antibody titres (IgG) in these sera were determined by enzyme-linked immunosorbent assay (ELISA), as described in Example 3.

5 The anti-RSV F IgG antibody response in the sera of the BALB/C mice are summarized in Figure 4. The mice immunized with the DNA construct, pMP44, had higher anti-F titres than the mice immunized with the SFV-RSV F RNA.

10 Two weeks after the second immunization, mice were challenged intranasally with 10^6 plaque forming units (pfu) of the A1 strain of RSV (BG-4A). Animals were sacrificed 4 days later. Lungs were aseptically removed, weighed, and homogenized in 2 mL of complete
15 culture medium. The virus titre in lung homogenates was determined in duplicate using vero cells, as previously described (ref. 41).

As seen in Table 2 below, immunization of mice with pMP44 DNA protected mice (5/6) against live RSV
20 challenge, in contrast to the lack of protection when immunization with SFV-RSV F RNA was effected. This result contrasts with the complete protection which is obtained using SFV-RSV F RNA as described in U.S. Patent Application Nos. 08/923,558, 08/476,397 and
25 08/896,500 where the results show protection after pretreatment with cardiotoxin.

Table 2

Group	Immunogen		Mean Virus Lung Titre	
	Prime	Boost	(log10/g \pm s.d)	% Protection
1	SFV-RSVF RNA	SFV-RSVF RNA	4.26	0
2	pMP44 DNA	pMP44DNA	2.12*	83

* Limit of detection = 1.8

EXAMPLE 4

This Example describes the determination of anti-
5 RSV F antibody titres.

Nunc-MaxiSorp plate wells were coated overnight at room temperature with 2.5 ng of immunoaffinity-purified RSV F protein diluted in 0.05M carbonate-bicarbonate buffer, pH 9.6. Wells were blocked for non-specific
10 binding by adding 0.1% BSA in PBS for 30 min. at room temperature, followed by two washes in a washing buffer of 0.1% BSA in PBS + 0.1% Tween 20. Serial two or four-fold dilutions of mouse serum was added to the wells. After a one hour incubation at room
15 temperature, plates were washed five times with washing buffer, and horseradish peroxidase (HRP) labeled conjugate was added at the appropriate optimal dilution in washing buffer. The total IgG assay used F(ab')₂ goat antimouse IgG (H+L specific)- HRP from Jackson
20 Immuno Research Laboratory Inc. (Baltimore, MD, USA). Sheep anti-mouse IgG1-HRP from Serotec (Toronto, Ontario, Canada) was used in the IgG1 assay and goat anti-mouse IgG2a from Caltag Laboratories (San Francisco, CA, USA) was used in the IgG2a assay.
25 Following one hour incubation at room temperature, the plates were washed five times with washing buffer, and hydrogen peroxide (substrate) in the presence of tetramethylbenzidine was added. The reaction was stopped by adding 2 M sulfuric acid. The colour was
30 read in a Multiscan Titertek plate reader at an optical density (OD) of 450 nm. The titre was taken as the reciprocal of the last dilution at which the OD was

approximately double. This OD must be greater than the negative control of the assay at the starting dilution. The pre-immune serum of each animal was used as the negative control.

5

SUMMARY OF THE DISCLOSURE

In summary of this disclosure, the present invention provides certain novel alphavirus derived DNA vectors containing genes encoding RSV F or RSV G proteins, or other paramyxovirus proteins, methods of immunization using such vectors and methods of diagnosis using such vectors. Modifications are possible within the scope of this invention.

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REFERENCES

1. McIntosh K. and Chanock R.M. in Fields B.N. and Knipe D.M. (eds). Virology. Raven Press, New York, 1990, pp.1045-1072.
5
2. Murphy B.R., Hall S.L., Kulkarni A.B., Crowe J.E., Collins P.L., Connors M., Karron R.A. and Chanock R.M., Virus Res 32, 13-36, 1994.
10
3. Osterweil D. and Norman D., Am Geriat Soc 36, 659-662, 1990.
4. Agius G., Dindinand G., Biggar R.J., Peyre R., Vaillant V., Ranger S., Poupet J.Y., Cisse M.F. and Casters M., J Med Virol 30, 117-127, 1990.
15
5. Katz S.L. in New vaccine development establishing priorities Vol 1. National Academic Press, Washington, 1985, pp. 3974 09.
20
6. Wertz G.W. and Sullender W.M., Biotechnology 20, 151-176, 1992 .
7. Fulginiti V.A., Eller J.J., Sieber O.F., Joyner J.W., Minamitani M. and Meiklejohn G., Am J Epidemiol 89, 449-463, 1969.
25
8. Chin J., Magoffin R.L., Shearer I.A., Schieble J.H. and Lennette E.H., Am J Epidemiol 89, 449-463, 1969.
30
9. Belshe R.B., Van Voris L.P. and Mufson M.A., J Infect Dis 145, 311-319, 1982.
35
10. Kim R.M., Arrobio J.O., Pyles G., Brandt C.D., Camargo E., Chanock R.M. and Parrott R.H., Pediatrics 48, 745-755, 1971.
11. Gruber C. and Levine S., J Gen Virol 64, 825-832, 1983.
40
12. Olmstead R.A., Elango N. and Prince G.A., Proc Natl Acad Sci USA 83, 7462-7466, 1991.
45

13. Parrington M., Cockle S., Wyde P., Du R.-P., Snell E., Yan W.-Y., Wang Q., Gisonni L., Sanhueza S., Ewasyshyn M. and Klein M., *Virus Genes* 14, 65-74, 1997
- 5 14. Fulginiti, V.A., Eller, J.J., Sieber, O.F., Joyner, J.W., Minamitani, M. and Meiklejohn, G. (1969) *Am. J. Epidemiol.* 89 (4), 435-448.
- 10 15. Chin, J., Magoffin, R.L., Shearer, L.A., Schieble, J.H. and Lennette, E.H. (1969) *Am. J. Epidemiol.* 89 (4), 449-463.
- 15 16. Jensen, K.E., Peeler, B.E. and Dulworth, W.G. (1962) *J. Immunol.* 89, 216-226.
- 20 17. Murphy, B.R., Prince, G.A., Collins, P.L., Van Wyke-Coelingh, K., Olmsted, R.A., Spriggs, M.K., Parrott, R.H., Kim, H.-Y., Brandt, C.D. and Chanock, R.M. (1988) *Vir. Res.* 11, 1-15.
- 25 18. Hall, S.L., Sarris, C.M., Tierney, E.L., London, W.T., and Murphy, B.R. (1993) *J. Infect. Dis.* 167, 958-962.
- 30 19. Belshe, R.B., Karron, R.A., Newman, F.K., Anderson, E.L., Nugent, S.L., Steinhoff, M., Clements, M.L., Wilson, M.H., Hall, S.L., Tierney, E.L. and Murphy, B.R. (1992) *J. Clin. Microbiol.* 30 (8), 2064-2070.
- 35 20. Hall, S.L., Stokes, A., Tierney, E.L., London, W.T., Belshe, R.B., Newman, F.C. and Murphy, B.R. (1992) *Vir. Res.* 22, 173-184.
- 40 21. Van Wyke Coelingh, K.L., Winter, C.C., Tierney, E.L., London, W.T. and Murphy, B.R. (1988) *J. Infect. Dis.* 157 (4), 655-662.
22. Ray, R., Novak, M., Duncan, J.D., Matsuoka, Y. and Compans, R.W. (1993) *J. Infect. Dis.* 167, 752-755.
23. Ray, R., Brown, V.E. and Compans, R.W. (1985) *J. Infect. Dis.* 152 (6), 1219-1230.
- 45 24. Ray, R. and Compans, R.W. (1987) *J. Gen. Virol.* 68, 409-418.

25. Ray, R., Glaze, B.J., Moldoveanu, Z. and Compans, R.W. (1988) J. Infect. Dis. 157 (4), 648-654.
- 5 26. Ray, R., Matsuoka, Y., Burnett, T.L., Glaze, B.J. and Compans, R.W. (1990) J. Infect. Dis. 162, 746-749.
- 10 27. Ray, R., Glaze, B.J. and Compans, R.W. (1988) J. Virol. 62 (3), 783-787.
28. Ewasyshyn, M., Caplan, B., Bonneau A.-M., Scollard, N., Graham, S., Usman, S. and Klein, M. (1992) Vaccine 10 (6), 412-420.
- 15 29. Ambrose, M.W., Wyde, P.R., Ewasyshyn, M., Bonneau, A.-M., Caplan, B., Meyer, H.L. and Klein, M. (1991) Vaccine 9, 505-511.
- 20 30. Kasel, J.A., Frank, A.L., Keitel, W.H., Taber, L.H., Glezen W.P. J. Virol. 1984; 52:828-32.
- 25 31. Lehman, D.J., Roof, L.L., Brideau, R.J., Aeed, P.A., Thomsen, D.R., Elhammer, A.P., Wathen, M.W. and Homa, F.L. (1993) J. Gen. Virol. 74, 459-469.
32. Brideau, R.J., Oien, N.L., Lehman, D.J., Homa, F.L. and Wathen, M.W. (1993) J. Gen. Virol. 74, 471-477.
- 30 33. Ebata, S.N., Prevec, L., Graham, F.L. and Dimock, K. (1992) Vir. Res. 24, 21-33.
- 35 34. Hall, S.L., Murphy, B.R. and Van Wyke Coelingh, K.L. (1991) Vaccine 9, 659-667.
- 35 35. Strauss E.G. and Strauss J.H., in Schlesinger S.S. and Schlesinger M.i. (eds). The Togaviridae and Flaviviridae. Plenum Press, New York, 1986, pp.35-90.
- 40 36. Chapman, B.S.; Thayer, R.M.; Vincent, K.A. and Haigwood, N.L., Nucl. Acids. Res. 1991, 19: 3979-3986.
- 45 37. Breathnack, R. and Harris, B.A., Nucl. Acids Res. 1983, 11: 7119-7136

38. Nabel, G.J. 1993, Proc. Natl. Acad. Sci. USA 90:
11307-11311.
- 5 39. Tang et al., Nature 1992, 356: 152-154
40. Furth et al. Analytical Biochemistry, 1992, 205:
365-368
- 10 41. Prince, G.A. et al, Am. J. Pathol. 93, 771 to 790,
1978.

CLAIMS

What we claim is:

1. A vector, comprising:
 - 5 a first DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complement of complete alphavirus RNA genome replication regions to permit *in vivo* replication; and
 - a second DNA sequence encoding a paramyxovirus
 - 10 protein or a protein fragment that generates antibodies that specifically react with the paramyxovirus protein, the second DNA sequence being inserted into a region of the first DNA sequence which is non-essential for replication, the first and second DNA sequences being
 - 15 under transcriptional control of a promoter.
2. The vector of claim 1 wherein the paramyxovirus protein is selected from the group consisting of a parainfluenza virus (PIV) and a respiratory syncytial
- 20 virus (RSV).
3. The vector of claim 2 wherein the PIV protein is selected from the group consisting of PIV-1, PIV-2, PIV-3 and PIV-4
- 25
4. The vector of claim 3 wherein said PIV protein is selected from the group consisting of the HN and F glycoproteins of PIV-3.

5. The vector of claim 4 wherein the RSV protein is selected from the group consisting of the F or G glycoprotein of RSV.
- 5 6. The vector of claim 1 wherein the second DNA sequence encodes a full length RSV F or RSV G proteins.
7. The vector of claim 1, wherein the second nucleotide sequence encodes a truncated RSV F or RSV G protein lacking the transmembrane anchor and cytoplasmic tail.
- 10 8. The vector of claim 1 wherein the alphavirus is a Semliki Forest virus.
- 15 9. The vector of claim 1 wherein the first DNA sequence is the Semliki Forest viral sequence contained in plasmid pSFVI.
- 20 10. The vector of claim 1 wherein the promoter sequence is an immediate early cytomegalovirus (CMV) promoter.
11. The vector of claim 1 further comprising a third DNA sequence located adjacent the second DNA sequence to enhance the immunoprotective ability of the paramyxovirus protein when expressed *in vivo* from the vector in a host.
- 25 12. The vector of claim 11 wherein the third nucleotide sequence comprises a pair of splice sites to prevent aberrant mRNA splicing, *in vivo* whereby substantially
- 30

35

all transcribed mRNA from the vector region
administration encodes the RSV protein.

13. The vector of claim 12 wherein the third nucleotide
5 sequence is located between the first nucleotide
sequence and the promoter sequence.

14. The vector of claim 13 wherein said third
nucleotide sequence is that of rabbit β -globin intron
10 II.

15. The vector of claim 10 wherein said promoter
sequence is an immediate early cytomegalovirus (CMV)
promoter and the human cytomegalovirus Intron A
15 sequence is provided downstream of the promoter and
upstream of the third nucleotide sequence.

16. The vector of claim 15 further comprising a fourth
nucleotide sequence at the 3'-end of the first
20 nucleotide sequence to to ensure proper *in vivo* cleavage
at the 3'-end of the first nucleotide sequence.

17. The vector of claim 16 wherein said fourth
nucleotide sequence is a hepatitis delta virus ribozyme
25 sequence.

18. The vector of claim 1 which has the identifying
characteristics of plasmid pMP44 shown in Figure 2D.

30 19. The vector of claim 1 which has SEQ ID No: 1.

20. A method of immunizing a host against disease caused by infection with paramyxovirus, which comprises administering to the host an effective amount of a vector as claimed in claim 1.

5

21. The method of claim 21 wherein said vector has the identifying characteristics of plasmid pMP44 shown in Figure 2D.

10 22. The method of claim 21 wherein said vector has SEQ ID no: 1.

23. A method of using a gene encoding an RSV F or G protein or a fragment of an RSV or G protein capable of
15 generating antibodies which specifically react with RSV F or G protein to protect a host against disease caused by infection with respiratory syncytial virus, which comprises:

isolating said gene;

20 operatively linking said gene to a DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complement of complete alphavirus RNA genome replication regions in a region of said DNA sequence which is non-essential for replication
25 to form a vector wherein said gene and DNA sequence are under transcriptional control of a promoter; and

introducing the vector into the host.

24. The method of claim 23 wherein said gene encoding
30 an RSV F protein encodes an RSV F protein lacking the transmembrane region.

25. The method of claim 24 wherein said promoter comprises the immediate early cytomegalovirus promoter.

5 26. The method of claim 25 including the step of:
operatively linking said gene to an immunoprotective enhancing sequence to produce an enhanced immunoprotection to said RSV F protein in said host.

10

27. The method of claim 26 wherein said immunoprotective enhancing sequence is introduced into said vector between said control sequence and said gene.

15 28. The method of claim 27 wherein said immunoprotection enhancing sequence comprises a pair of splice sites to prevent aberrant mRNA splicing whereby substantially intact transcribed mRNA encodes an RSV F protein.

20

29. The method of claim 28 wherein said immunoprotection enhancing sequence is that of rabbit β -globin intron II.

25 30. The method of claim 23 wherein said vector is plasmid pMP44.

31. The vector of claim 23 wherein said vector has SEQ ID no: 1.

30

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32. A method of producing a vaccine for protection of a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises:

isolating a first DNA sequence encoding an RSV or G protein, from which the transmembrane anchor and cytoplasmic tail may be absent;

operatively linking said first DNA sequence to a second DNA sequence which is complementary to at least part of an alphavirus RNA genome and having the complete alphavirus genome replication regions in a region of said second DNA sequence which is non-essential for replication to form a vector wherein said first and second DNA sequences are under transcriptional control of a promoter; and

formulating the vector as a vaccine for *in vivo* administration.

33. The composition of claim 32 wherein said vector has the identifying characteristics of pMP44 shown in Figure 2D.

34. The method of claim 32 wherein said vector has SEQ ID no: 1.

35. A vaccine for administration to a host, including a human host, produced by the method of claim 32.

36. An immunogenic composition comprising an immunoeffective amount of a vector of claim 1.

30

39

37. The composition of claim 36 wherein said vector has the identifying characteristic of pMP44 in Figure 2D.

38. The composition of claim 36 wherein said vector has

5 SEQ ID no: 1.

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⁴¹
Figure 4 Construction of pMP44

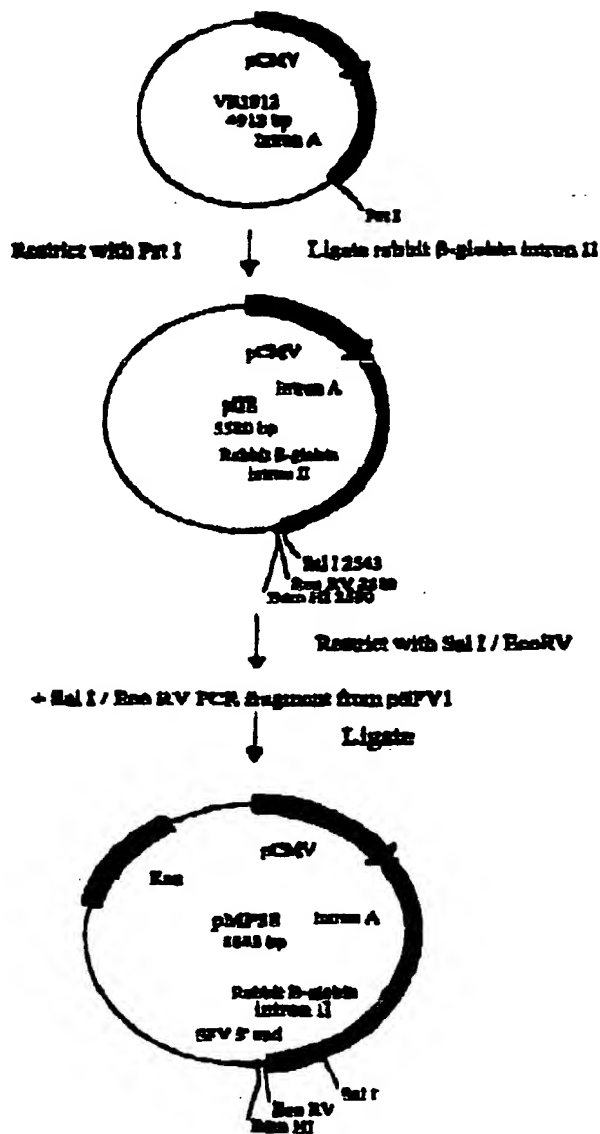


FIG. 4a 1a

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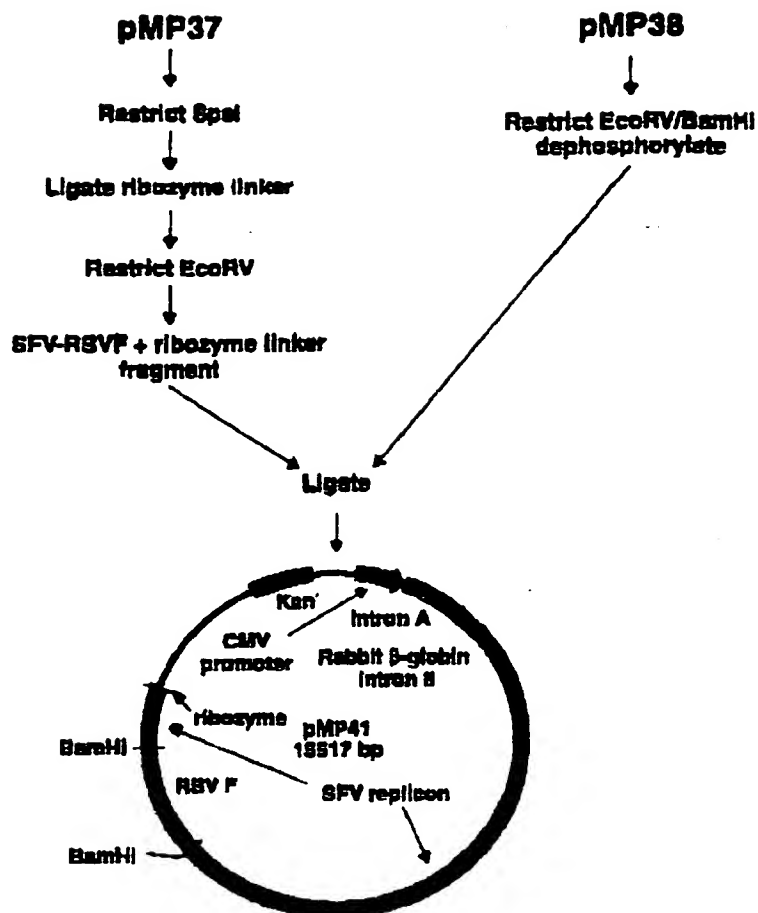
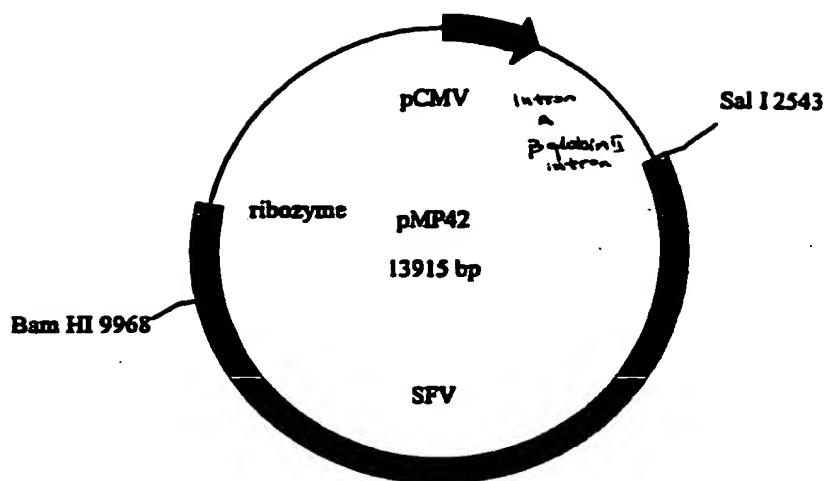


Fig 1B

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+ Ben HI fragment
from pM137

L. 5. 12

F12 1C

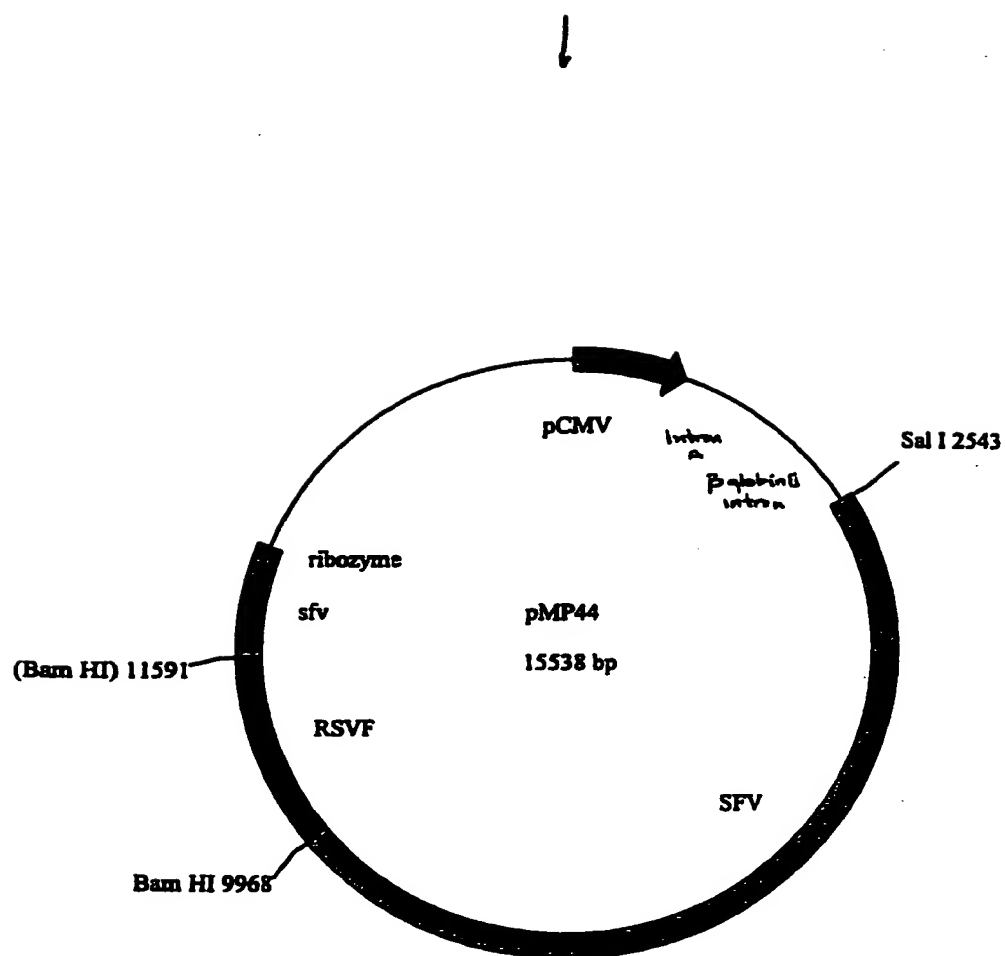


FIG 10

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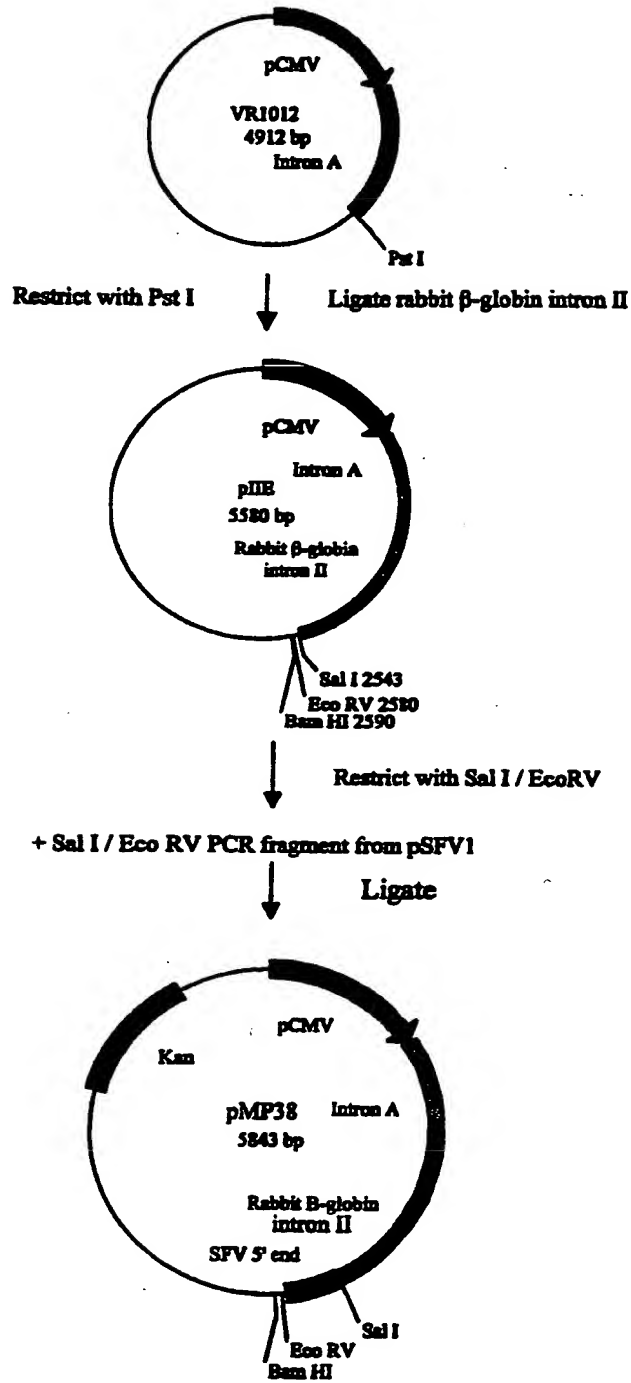
Figure 1 Construction of pMP44

fig. 2A

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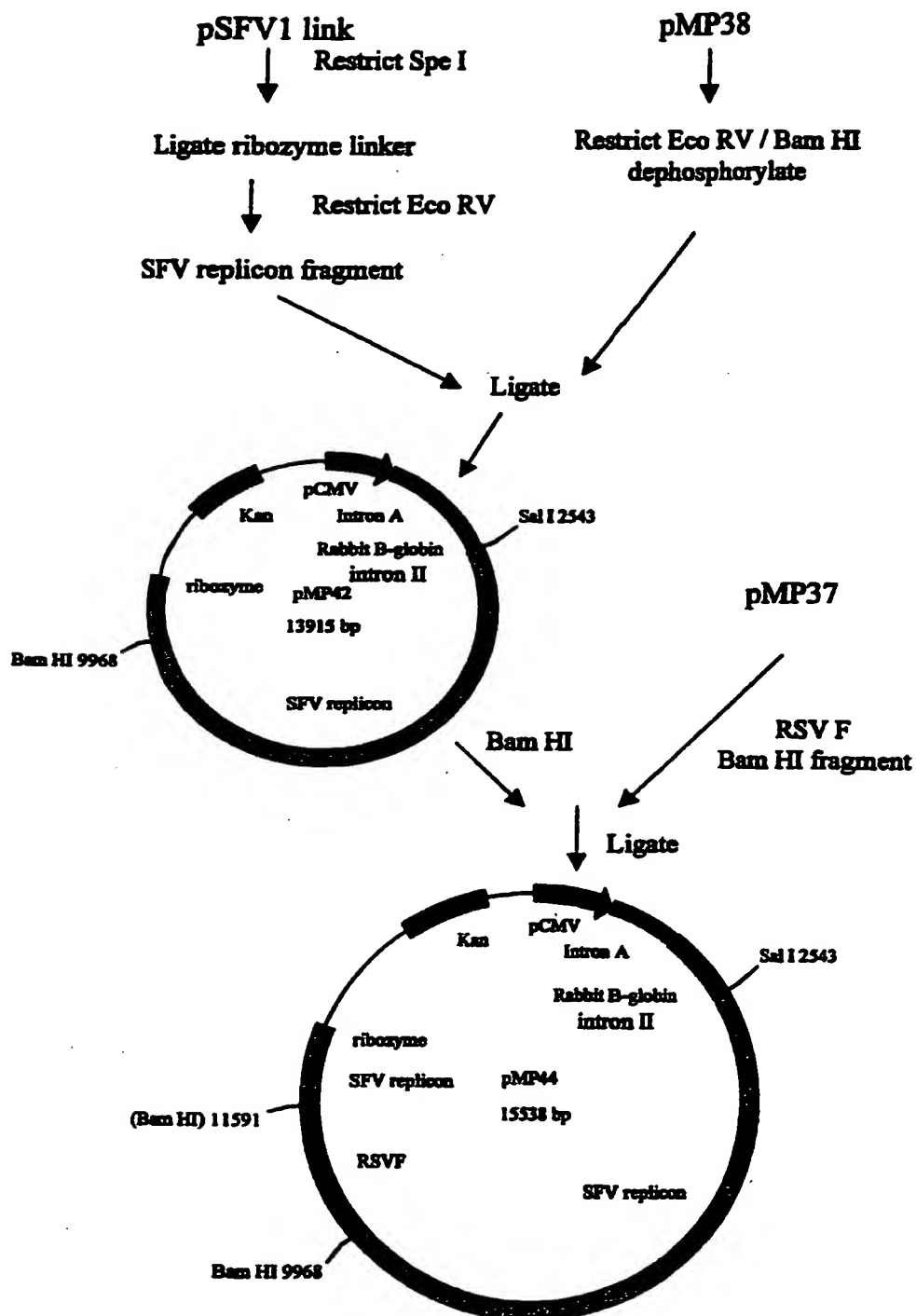


Fig. 2A

Figure 2 Nucleotide sequence of plasmid pMP44

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tcgcgcgttt  cggatgatgac  ggtgaaaacc  tctgacacat  gcagctcccc  gagacgggtca  60
cagcttgtct  gtaagcggat  gccgggagca  gacaagcccc  tcagggcgcg  tcagcgggtg  120
ttggcgggtg  tcggggctgg  ctttaactatg  cggcatcaga  gcagattgta  ctgagagtgc  180
accatatgcg  gtgtgaaata  ccgcacagat  gcgtaaggag  aaaataccgc  atcagattgg  240
ctattggcca  ttgcatacgt  tgtatccata  tcataatatg  tacatttata  ttggctcatg  300
tccaacatta  ccgccatgtt  gacattgatt  attgactagt  tattaatagt  aatcaattac  360
ggggtcatta  gttcatagcc  catatatgga  gttccgcgtt  acataactta  cggtaaatgg  420
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catagtaacg  ccaataggga  ctttccattg  acgtcaatgg  gtggagtatt  tacggtaaac  540
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Figure 4. Anti-RSV F titres in sera from mice taken 4 weeks after priming and 2 weeks after boosting

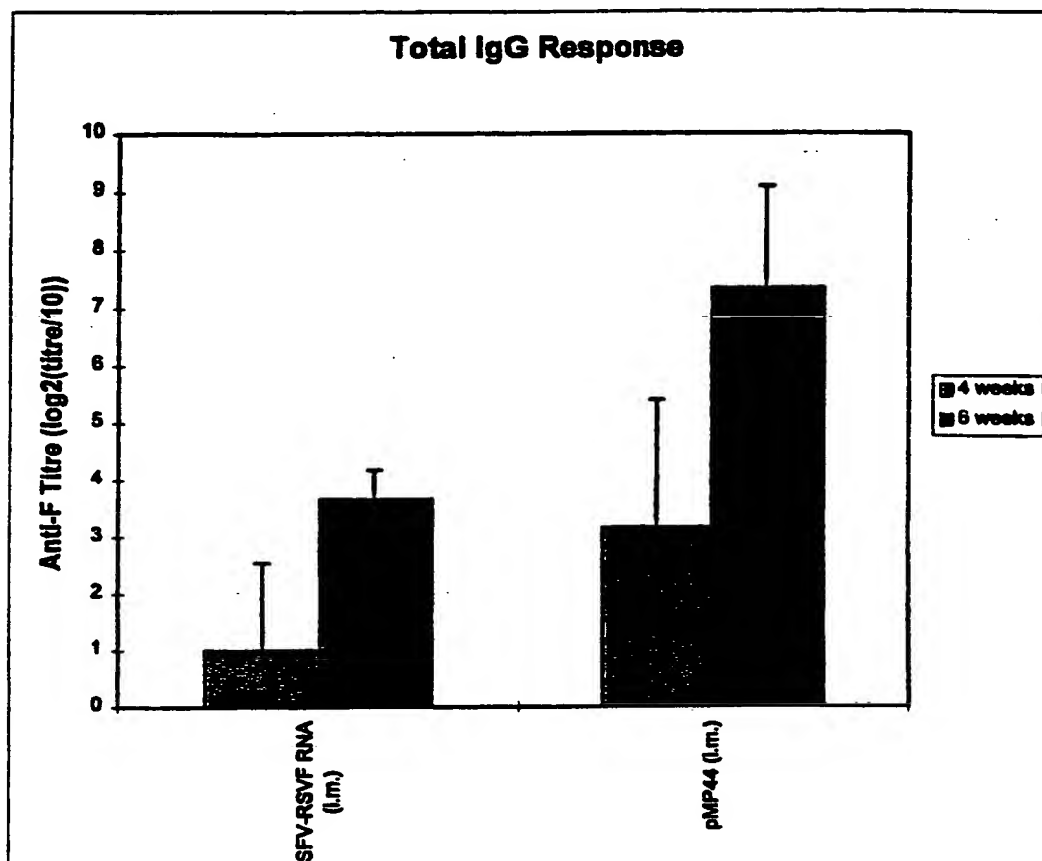


Figure 5 Ribozyme linker for pMP42

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GCCCAGCCGTACCGTAGAGGTGAGGAGCGCCAGGCTGAGTGGACCCGTAGGCTTCCTCCTCGTGCAGGTGAGCCTACCGATTCCCTCTCTAG
3'

Figure 6 ASFV Eco RV-Spe I fragment ligated to ribozyme

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INTERNATIONAL SEARCH REPORT

Inter. .onal Application No

PCT/CA 98/01064

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/86 C12N15/45 C07K14/135 C07K14/115 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 40945 A (CONNAUGHT LAB ;LI XIAOMAO (CA); EWASYSHYN MARY E (CA); SAMBHARA SU) 19 December 1996 cited in the application see the whole document, especially page 6, lines 2-9; page 14, lines 15-21; and page 23, lines 18-23	1-36
Y	WO 95 27044 A (BIOPTION AB ;LILJESTROEM PETER (SE); GAROFF HENRIK (SE)) 12 October 1995 cited in the application see the whole document, especially page 8, lines 12-22	1-36
A	WO 96 17072 A (VIAGENE INC) 6 June 1996 see the whole document	1-36
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

23 April 1999

Date of mailing of the international search report

03/05/1999

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Mandl, B

INTERNATIONAL SEARCH REPORT

Inter. Appl. No.

PCT/CA 98/01064

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZHOU X. ET AL.: "Self-replicating Semliki-Forest virus RNA as recombinant vaccine" VACCINE, vol. 12, no. 16, 1994, pages 1510-1514, XP002089524 cited in the application see the whole document ---	1-36
A	LILJESTROEM P. ET AL.: "A NEW GENERATION OF ANIMAL CELL EXPRESSION VECTORS BASED ON THE SEMLIKI FOREST VIRUS REPLICON" BIO/TECHNOLOGY, vol. 9, December 1991, pages 1356-1361, XP000616021 cited in the application see the whole document ---	1-36
E,L	WO 99 11808 A (CONNAUGHT LAB ;PARRINGTON MARK (CA)) 11 March 1999 cited in the application see the whole document -----	1-9,20, 23,24, 32,35,36

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/00840

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/86 C12N15/45 C07K14/115 C07K14/135 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 40945 A (CONNAUGHT LAB ;LI XIAOMAO (CA); EWASYSHYN MARY E (CA); SAMBHARA SU) 19 December 1996 cited in the application see the whole document ---	1-53
Y	LILJESTROEM P. ET AL.: "A NEW GENERATION OF ANIMAL CELL EXPRESSION VECTORS BASED ON THE SEMLIKI FOREST VIRUS REPLICON" BIO/TECHNOLOGY, vol. 9, December 1991, pages 1356-1361, XP000616021 cited in the application see the whole document ---	1-53
A	US 5 614 372 A (LILJA HANS ET AL) 25 March 1997 see column 5, line 61 - line 65 ---	1-53

-/--

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

8 January 1999

Date of mailing of the international search report

22/01/1999

Name and mailing address of the ISA

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Authorized officer

Mandl, B

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 98/00840

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>SCHLESINGER S.: "ALPHAVIRUSES - VECTORS FOR THE EXPRESSION OF HETEROLOGOUS GENES" TRENDS IN BIOTECHNOLOGY, vol. 11, no. 1, 1 January 1993, pages 18-22, XP000358625 see page 20, left-hand column, last paragraph - right-hand column, paragraph 1 -----</p>	1-53
A	<p>WO 96 17072 A (VIAGENE INC) 6 June 1996 see the whole document, especially page 38, lines 20-31 and page 61 lines 12-32 -----</p>	1-53
A	<p>ZHOU X. ET AL.: "Self-replicating Semliki-Forest virus RNA as recombinant vaccine." VACCINE, vol. 12, no. 16, 1994, pages 1510-1514, XP002089524 cited in the application see the whole document -----</p>	1-53

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 98/00840

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 45-49
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
information on patent family members

International Application No
PCT/CA 98/00840

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9640945 A	19-12-1996	AU 695527 B	13-08-1998
		AU 6117696 A	30-12-1996
		CA 2223610 A	19-12-1996
		EP 0832253 A	01-04-1998
		US 5843913 A	01-12-1998
US 5614372 A	25-03-1997	EP 0811164 A	10-12-1997
		WO 9626442 A	29-08-1996
WO 9617072 A	06-06-1996	AU 4594996 A	19-06-1996
		EP 0797679 A	01-10-1997
		US 5814482 A	29-09-1998
		US 5843723 A	01-12-1998
		US 5789245 A	04-08-1998

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 98/01064

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 20-30
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 98/01064

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9640945 A	19-12-1996	AU 695527 B AU 6117696 A CA 2223610 A EP 0832253 A US 5843913 A US 5880104 A	13-08-1998 30-12-1996 19-12-1996 01-04-1998 01-12-1998 09-03-1999
WO 9527044 A	12-10-1995	AU 699384 B AU 2155795 A CA 2184261 A EP 0753053 A FI 963860 A JP 9511143 T	03-12-1998 23-10-1995 12-10-1995 15-01-1997 27-09-1996 11-11-1997
WO 9617072 A	06-06-1996	AU 4594996 A EP 0797679 A US 5814482 A US 5843723 A US 5789245 A	19-06-1996 01-10-1997 29-09-1998 01-12-1998 04-08-1998
WO 9911808 A	11-03-1999	NONE	